Editorials

Why Does the Blood Not Coagulate?

A BRITISH PHYSICIAN, T. W. Jones, asked 150 years ago, "Why does the blood circulating in the vessels not coagulate?"[1] We still seek an answer to this logical question. Even children know that when they cut themselves the blood that oozes from the wound quickly clots. Why does it wait to do this until it has left the injured blood vessel? Innumerable theories have been offered in explanation.[2]

A host of experimental work, some of it recounted skillfully elsewhere in this issue by Samuel Rapaport, MD,[3] teaches us the steps that lead to coagulation when blood is withdrawn from the body into the artificial world of test tubes. As Lister appreciated more than a century ago,[4] one way that the clotting process may begin is that shed blood touches a "foreign" surface, that is, a surface other than the normal endothelial lining of the vascular tree. The initiation of clotting in this manner is described as the intrinsic pathway of thrombin formation. Alternatively, as described long ago by Thackrah,[5] clotting also comes about when blood comes into contact with injured tissues. In modern terms, a clot-promoting agent furnished by injured tissues, variously called tissue factor or tissue thromboplastin, forms a complex with a plasma protein, factor VII, that then initiates thrombin formation through reactions of the extrinsic pathway of clotting. The terminology is confusing, as in each case something extrinsic to the blood is responsible for starting the clotting process.

A friend of mine was fond of saying, "It is we who are simple, not nature." Dividing the steps leading to thrombin formation into intrinsic and extrinsic pathways makes it easier to draw diagrams, but biology is seldom that accommodating. When Rapaport and co-workers showed that the prothrombin time was shortened on exposing plasma to glass,[6] it was evident that the separation of the two pathways was mostly in the mind of the observer. When plasma comes into contact with glass, this foreign surface activates Hageman factor (factor XII), and activated Hageman factor, it turned out, can change factor VII to its enzymatically active state.[7,8] In the years since these observations were published, seemingly innumerable examples of cross-reactions between the intrinsic and extrinsic pathways have been found, some described in Rapaport's review.

If this tells us how blood can coagulate, it does little to answer Jones's question. The logic of biology suggests that the potent reactions leading to the formation of a clot cannot go unchecked; the alternative would be unbridled intravascular clotting. Some early experimental observations offering an explanation for modulation of the clotting process were described by Morawitz and Rettger, who found that plasma itself could inactivate thrombin, protecting circulating blood from the inadvertent formation of this enzyme.[9,10] This agent, antithrombin III (or just "antithrombin"), is of peculiar clinical interest. For the most part, the anticoagulant action of heparin depends on its ability to form a complex with antithrombin III. Antithrombin III bound to heparin inactivates not only thrombin but also factor Xa (activated Stuart factor), factor IXa (activated Christmas factor), factor XIa (activated plasma thromboplastin antecedent), and factor XIIa (activated Hageman factor). Heparin and the heparan proteoglycan of blood vessel walls also combine with another plasma protein, heparin cofactor II, to form a second inhibitor of thrombin, a further check against the formation of fibrin.

Other plasma proteins provide still other controls over the steps of the intrinsic pathway of thrombin formation. $C\bar{1}$ inhibitor blocks the enzymatic activity not only of the first component of complement but also of factor XIIa, factor XIa, and plasmin. α_1-Antitrypsin inhibits factor XIa. And protein C, a protease that is activated by thrombin and abetted by a nonenzymatic cofactor, protein S, inactivates the thrombin-altered forms of both factor VIII (antihemophilic factor) and factor V (proaccelerin).

A different class of inhibitors of clotting has had some recent attention. Suspensions of platelets or of nucleated peripheral blood cells and the supernates of such suspensions inhibit the activation of Hageman factor.[11,12] The agent or agents responsible have not yet been identified but seem different from other described inhibitors of clotting. Even more exciting, the supernates of cultured human and bovine vascular endothelial cells and the cytoplasmic fluid of human vascular endothelial cells inhibit the activation of Hageman factor.[13,14] These inhibitors make "sense" teleologically, for they protect the intrinsic pathway of thrombin formation from inadvertent activation by these biologically active cells. That this hypothesis may have merit comes from an examination of the odd fact that filariae or schistosomes residing in human blood vessels do not seem to stimulate the growth of thrombi. In both cases, the offending organisms appear to be covered on their surfaces with inhibitors of the activation of Hageman factor.[15,16]

In his review, Rapaport summarizes what is now known about yet another controller of clotting, tissue factor pathway inhibitor (née extrinsic pathway inhibitor or lipoprotein-associated inhibitor). This protein has a curious history, some of it reviewed by Broze and Miletich and by Sandset and Abildgaard.[17,18] In 1919 Obata, trying to understand the pathogenesis of eclampsia, injected saline extracts of human placenta into mice.[19] These animals had convulsions, became comatose, and died within minutes. These experiments seemed a replay of those of de Blainville and Wooldridge, whose similar studies laid the groundwork for our current understanding of disseminated intravascular coagulation.[20,21] But Obata carried his studies further. When the placental extract was incubated with serum before it was injected, it appeared to lose its toxicity. Confirming these experiments, Schneider found that the agent in serum that inhibited the toxicity of placental extracts was heat-labile.[22,23] At the same time, Thomas reported that the intravenous injection of brain or lung tissue into mice induced ataxia and convulsions, observations reminiscent of those of Obata.[24,25] Thomas, too, found that mammalian serum inhibited the toxic effect of these tissue extracts. The effect of serum was dependent on the presence of calcium ions and was abolished by heating the serum at 65°C for 30 minutes. Thomas also noted that the inhibitory property of serum could be adsorbed to the tissue particles. Thus, by 1947 it was clear that serum contained an inhibitory activity that would neutralize what is now called tissue factor or tissue thromboplastin, an interpretation not lost to these early investigators.

Several groups of investigators have extended these ear-

lier studies.[17,26,27] In his review, Rapaport clearly summarizes what is known about tissue factor pathway inhibitor. This inhibitor successively binds factors Xa and VIIa, effectively reducing the generation of thrombin. What makes these observations particularly exciting is evidence that, in animals, depletion of this inhibitor may enhance disseminated intravascular coagulation. Rapaport's speculations about this inhibitor's role in the control of disseminated intravascular coagulation find support in experiments of Day and colleagues.[28] These investigators showed that administering recombinant tissue factor pathway inhibitor suppressed the induction of disseminated intravascular coagulation in rabbits infused with tissue thromboplastin, bringing a modern explanation to the observations of Obata and those who followed. It is not hard to speculate that recombinant tissue factor pathway inhibitor may find therapeutic use in patients with disseminated intravascular coagulation.

All this would be esoteric were it not for the fact that hereditary deficiencies of one or another of the inhibitors of clotting may be complicated by serious pathologic changes. Thus, deficiencies of antithrombin III, heparin cofactor II, or proteins C or S are accompanied by a tendency to recurrent thrombosis.[29] But patients with one or another of these deficiencies provide a puzzle not yet clearly addressed. For example, hereditary partial deficiency of antithrombin III is lifelong. From time to time, perhaps at intervals of many years, patients who have this heterozygous deficiency of antithrombin III may sustain thrombosis. I assume that the initiation of thrombosis takes but a short time. Why is it these patients do not have continual, major intravascular clotting? Another enigma surrounds the deficiency of $C\bar{1}$ inhibitor, a protein that inhibits both clotting and fibrinolysis. A deficiency of $C\bar{1}$ inhibitor is not accompanied by a thrombotic tendency, but rather is the pathogenetic feature of hereditary angioneurotic edema.[30] Sometimes nature tells us what are important biologic reactions, regardless of what we think we know from laboratory studies.

Another puzzle close to my heart is the persistence of Hageman factor throughout the mammalian kingdom, even though its absence does not seem to impair life. This seems to violate the principle that useless systems are cast off during the course of evolution. If this principle has merit, what secret need is there for Hageman factor, and, if this is true, why is the absence of Hageman factor not a handicap?

And then there is the unsettling intertwining of the reactions of clotting, fibrinolysis, complement, and inflammation. Some years ago, when I tried to write a synthesis of what I knew about blood clotting, I found that, try as I would, I could not separate what I knew about coagulation from what I thought I knew about these other systems.[31] Trained to look at each of these systems as a discrete entity, I found it difficult to accept that in real life the reactions of these four systems are hopelessly entangled. Like all great revelations, it did not take long to find that my views were not the news of the week; yet my unitarian synthesis offended a scientific bureaucracy I did not even know existed.

A good review should stimulate readers to think not just about problems solved but about questions not yet answered. Rapaport's review fits this bill.

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The Final Career

"RETIREMENT" has many definitions. The third offering in the *American Heritage Dictionary* says, "To withdraw from business or public life so as to live at leisure on one's income, savings or pension." It reverberates with doom—a doleful prescription for an early demise.

Because the editors were kind enough to ask me to comment on the subject, presented so tidily and provocatively by Virshup and Coombs[1]—I am a novice retiree . . . a mere 13